PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/002052	26.02.2005	01.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12N15/82, C12N5/10, A01H5/00

Applicant
BASF PLANT SCIENCE GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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International application No.
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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 i

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-6
	No:	Claims	1, 7-21
Inventive step (IS)	Yes:	Claims	2-6
	No:	Claims	1, 7-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	-

2. Citations and explanations

see separate sheet

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Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

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The present application pertains to promoters for the expression of genes of interest in vegetative tissue, but not in seeds.

Re Item IV
Lack of unity of invention

The application lacks unity as required by Article 3(4)(iii) PCT and Rule 13 PCT:

Rule 13.1 PCT states that for unity of invention to be present, all subject-matter should be linked by a single general inventive concept.

The present application contains two groups as indicated in the search report:

Group 1: Claims 1 (partially), 2, 3, 7-21 (all partially)
Pea ptxA gene promoter represented by Seq. ID No. 1 and its use
Group 2: Claims 1 (partially), 4-6, 7-21 (all partially)
Soybean SbHRGP3 gene promoter represented by Seq. ID Nos. 2 and 7-9 and its use

The common concept linking these two groups is that the claimed promoters lead to a nearly identical expression pattern wherein genes of interest are expressed in vegetative tissue, but not in seeds.
However, this concept is not novel, as such methods have been disclosed (see D2 and D3, also referred to in item V, below). As a common concept linking different groups in one application has to be novel and inventive, the present concept does not represent an acceptable link concerning unity of invention.

Since no further special technical feature (Rule 13.2 PCT) could be identified to provide a linking concept between the different groups of the invention, each of these groups must be regarded as a separate invention.

It has to be noted that D2 as well as D3 pertain to promoters expressed in vegetative tissue. D2 discloses a banana actin 1 promoter leading to expression in pseudo-stems, leaves and roots, and D3 discloses an OsGA3ox2 promoter leading to expression in

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vegetative growth tissue, in particular in stems and leaves. In both documents the expression in flowers, fruits and seeds has not been examined. Consequently, the objection as to lack of unity is based on the assumption that the promoters of D2 and D3 indeed are not expressed in seeds. In case that the applicant provides comparative data or other evidence that these promoters are expressed in seeds, the objection as to lack of unity will not be maintained.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: AHN JI HOON ET AL: "A novel extensin gene encoding a hydroxyproline-rich glycoprotein requires sucrose for its wound-inducible expression in transgenic plants" PLANT CELL, vol. 8, no. 9, 1996, pages 1477-1490, XP002340753 ISSN: 1040-4651

D2: HERMANN S R ET AL: "The banana actin 1 promoter drives near-constitutive transgene expression in vegetative tissues of banana (Musa spp.)" PLANT CELL REPORTS, vol. 20, no. 6, September 2001 (2001-09), pages 525-530, XP002340754 ISSN: 0721-7714

D3: EP-A-1 375 668 (NATIONAL INSTITUTE OF AGROBIOLOGICAL SCIENCES) 2 January 2004 (2004-01-02)

D4: DATABASE EMBL [Online] 29 October 1997 (1997-10-29), "Pisum sativum ptxA gene" XP002340760 retrieved from EBI accession no. EM_PRO:PSPTXAG Database accession no. PSPTXAG

D5: AHN JI HOON ET AL: "Expression of a soybean hydroxyproline-rich glycoprotein gene is correlated with maturation of roots" PLANT PHYSIOLOGY (ROCKVILLE), vol. 116, no. 2, February 1998 (1998-02), pages 671-679, XP002340755 ISSN: 0032-0889

D6: SADANANDOM ARIVANANTHAN ET AL: "Identification of a peptide methionine sulphoxide reductase gene in a oleosin promoter from Brassica napus" PLANT JOURNAL, vol. 10, no. 2, 1996, pages 235-242, XP002340756 ISSN: 0960-7412

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1. Document D2 discloses the banana actin 1 (ACT1) promoter. The ACT1 gene and its deduced amino acid sequence show highest sequence identity with vegetatively expressed actin genes (see abstract). According to northern analyses, expression can be found in leaves, roots and flower parts (see abstract). In transgenic banana plants, the ACT1 promoter drives strong expression in pseudo-stems, leaves and roots (p. 529). Data for flowers, fruits and seeds are not disclosed. In the absence of evidence of expression in seeds, the promoter of D2 is considered to be a functional equivalent which renders claims 1 and 7-21 not novel (Article 33(2) PCT). Novelty of claims 2-6 cannot be finally assessed due to clarity problems (see item VIII, below).

2. Document D3 discloses the OsGA3ox2 gene promoter specifically expressing exogenous genes in vegetative growth tissue, and in particular in leaves and stems (see abstract). In view of D3 claims 1 and 7-21 have to be considered as not complying with the requirements of Article 33(2) PCT. Novelty of claims 2-6 cannot be finally assessed due to clarity problems (see item VIII, below).

   The objections with respect to novelty will not be maintained in case that the applicant provides comparative data or other evidence that the disclosures of D2 and D3 do not fall within the scope of present claims 1-21.

3. D1 is a scientific publication dealing with the identification of the SbHRGP3 gene and promoter-GUS fusions expressed in transgenic tobacco (see abstract, p. 1481 and p. 1488). For the promoter-GUS constructs a 922 bp fragment of the promoter was used (p. 1488). It drove expression in leaves and stems upon wounding in combination with sucrose (p. 1482) and was not expressed in roots (p. 1482). In seedlings expression could be observed at days 3 and 6-8.

3.1 This disclosure is in marked contrast to the teachings of the present application. As claims 1-21 are functionally limited and the construct of D1 does not show expression in roots (neither with or without wounding and/or sucrose), novelty has to be

acknowledged for claims 1-21 in view of D1. However, objections under Article 5 and 6 PCT with respect to general disclosure and support have to raised.

3.2 Claims 1 and 4-21 relate to an expression construct comprising the SbHRGP3 promoter or functional equivalent fragments or homologues. A length of the promoter, the fragments or homologues is not indicated and a test method to unambiguously characterize the expression is not given. In view of the different expression of the fragment disclosed in D1 it is considered that the claimed constructs do not solve the underlying problem over the whole range claimed. It is even questionable if any fragment or homolog solves the underlying problem. The subject-matter therefore does not comply with Articles 5 and 6 PCT.
The scope of the claims therefore has to be limited to promoters for which experimental data have been or will be provided with respect to the claimed expression pattern. As a consequence thereof, also formulations such as "hybridizing under high stringency conditions" are not considered suitable to characterize the subject-matter. This also applies to present claim 2.

Re Item VII
Certain defects in the international application

4. According to Rule 5.1(a)(ii) PCT background art useful for understanding, searching and examination of the invention should be cited in the description. A reference to D1 should therefore be introduced into the description.

Re Item VIII
Certain observations on the international application

5. The formulations "functional equivalent fragments" and "functional equivalent homologues" as used in claims 1-6 and 16 are considered unclear (Article 6 PCT). These formulations leave the skilled person in doubt about the exact scope of the claim.
Furthermore, the term "homolog" describes a relation based on evolution. A relation

based on the sequence should be expressed by sequence identity with respect to a particular Seq. ID No.

6. Claims 1, 2, 4 and 16 encompass complements of the promoter sequence. On page 29 of the description it is stated where potential TATA boxes of Seq. ID Nos. 1 and 2 are localized. As outlined in D6, page 235 (Introduction), the unidirectionality of promoters is conferred by the TATA box. It is therefore considered that the claimed promoters are unidirectional and that consequently the claimed complements do not solve the underlying problem (Articles 5 and 6 PCT). In addition, the claimed complements lead to objections under Rule 13.1 PCT with respect to unity of invention.

7. The term "predominant" in claims 1 and 16 does not delimit the scope of the claims sufficiently from the prior art (Article 6 PCT). A promoter having low expression in seeds and high expression in leaves can be considered as predominantly expressing in vegetative plant tissues. Such promoters are known in the art, thus rendering claims 1 and 16 not novel.

8. In addition, the term "vegetative plant tissue" also lacks clarity (Article 6 PCT) with respect to the claimed subject-matter. Vegetative tissues are those tissues present in a plant during the vegetative state. Upon induction to flowering, reproductive organs (i.e. flowers) and after pollination seeds/fruits are formed. In their present formulation claims 1 and 16 do not make clear that the claimed promoters are not expressed in seeds.

9. Claims 3 and 5 pertain to fragments "from about base pair... to about base pair...." This renders claims 3 and 5 unclear (Article 6 PCT) as a skilled person will not know which sequences fall into the scope of the claims.
As a definition, a variance of 20 percent up or down is given on page 9 of the description. This is far beyond what a skilled person might envisage so that the claim needs clarification.

10. According to claim 1 the claimed expression construct leads to expression in vegetative tissues. In claims 12 and 13 other transgenic organisms such as bacteria,

yeast, fungi and animals are encompassed in the present wording of the claims. This is considered unclear (Article 6 PCT), particularly in view of the fact that the claimed organisms can be produced, but the claimed expression of the construct will under no circumstance be obtained as the relevant organs are not present.

11. The formulation "a cell culture, part or transgenic propagation material derived from a transgenic organism..." in claim 15 is unclear as such materials, even if derived from transgenic organisms are not necessarily transgenic or carry the same transgene. Furthermore, this formulation does not contain any technical information. Technical information might for example be provided by identifying the sequence(s) that have to be contained in the material.

12. The present claims in general pertain to expression in monocotyledonous and dicotyledonous plants. Experimental data concerning the expression have only been provided for Arabidopsis and canola, but not for any monocotyledonous plant. As it is known in the art that promoters are expressed differently in mono- and dicotyledonous plants, for reasons of clarity (Article 6 PCT) either experimental data for monocotyledonous plants have to be provided or the claims have to be reformulated.